Utilization of Neutral Glycerides and Phosphatidylethanolamine by the Phospholipase A₁ of the Plasma Membranes of Rat Liver*

MOSELEY WAITE‡ AND PATRICIA SISSON

From the Department of Biochemistry, The Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, North Carolina 27103

SUMMARY

The phospholipase A₁ solubilized by heparin from the plasma membranes of rat liver can catalyze the hydrolysis of monoglyceride, diglyceride, and monoaoylglycerophosphorylethanolamine in addition to phosphatidylethanolamine. Furthermore, the enzyme catalyzes the transacylation of the acyl group from position 1 of these glycerides to an acceptor monoglyceride to form diglyceride. Triton X-100 which disperses the lipids reduces transacylation and favors the utilization of water as the acyl acceptor (hydrolysis). Since free fatty acid is not incorporated, the transacylation appears to be direct rather than the combination of hydrolysis and reacylation. The influence of Ca²⁺ on transacylation and hydrolysis was dependent upon the mixtures of lipids used as substrates. Co-elution of the hydrolytic and transacylation activities from a column of Sephadex G-200 and heat inactivation of all activities indicate that a single enzyme catalyzes both reactions. It is tentatively suggested this enzyme be more appropriately named monoglyceride acyltransferase since monoglyceride is the preferred substrate and transacylation is the predominating reaction.

Hydrolysis of phospholipids by preparation of plasma membranes from liver has been demonstrated in four laboratories (1-4). Recent evidence showed that both phospholipase A₁ (EC 3.1.1.4) which was most active on phosphatidylylycerol and phospholipase A₂ which acted most rapidly on phosphatidylglycerol exist in plasma membranes (5). Zieve and Zieve (6) suggested that the plasma membranes of liver might be the source of the serum post-heparin phospholipase. We therefore studied the effect of heparin on the phospholipase A₁ of the plasma membranes and found that the phospholipase A₁ could be solubilized from the plasma membranes by heparin. Furthermore, there was a direct correlation between the amount of heparin required for stimulation of the phospholipase A₁ and the amount of [³⁵S]heparin bound to the membrane. The enzyme, once freed from the membrane, no longer had an absolute requirement for Ca²⁺.

In this report we describe the activity of the heparin-solubilized phospholipase A₁ on phosphatidylethanolamine, monoaoylglycerophosphorylethanolamine, and the neutral glycerides. Monoglyceride and diglyceride were hydrolyzed at rates faster than phosphatidylethanolamine. In addition to hydrolysis (Reactions 1 to 3, Scheme 1) the enzyme catalyzed a transacylation of the fatty acid of position 1 of either monoglyceride or diglyceride (Reaction 4) or phosphatidylethanolamine (Reaction 5) to an acceptor monoglyceride. Vogel et al. (9) first demonstrated that this enzyme could transacylate with various water-soluble alcohols as acyl acceptors.

EXPERIMENTAL PROCEDURES

Methods—The soluble fraction from the plasma membranes was prepared by treatment with heparin as described earlier (8). Lipolytic activity was measured using 50 μM of each lipid added
as an ultrasonic suspension in water, 100 mM Tris-HCl, pH 9.0, 5 mM CaCl₂, and 40 to 60 μg of the soluble protein in a total volume of 1.0 ml. The incubation time was 10 min and the temperature was 37°C. The lipids, when used as a mixture, were prepared as ultrasonic suspension separately or in combination; no difference in the results was found. Any variations in the standard conditions are noted in the individual experiments. The reaction products were separated by thin layer chromatography in the ether-heptane-formic acid system (35:65:1.5 by volume) which separates triglyceride, fatty acid, 1,3-diglyceride, and 1,2-diglyceride, and monoacylglycerol and phospholipid (both at or near the origin), or in the chloroform-lignin (b.p. 63-75°C)-acetic acid (70:30:4 by volume) followed by chloroform-metha- nol-water (70:30:4 by volume) which separates the neutral lipid, phosphatidylethanolamine, and monoacylglycerophosphorylethanolamine. Diglycerides which contained different numbers of double bonds in the acyl groups were separated on thin layer plates which were made with 5% AgNO₃ (weight per volume of H₂O). The developing system was chloroform-ethanol (95:5 by volume) (10). The products were made visible by staining with I₂ vapor and their radioactivity was determined by counting the compound on silica acid in a scintillation fluid or near the origin), or in the chloroform-lignin (b.p. 63-75°C)-acetic acid (70:30:4 by volume) followed by chloroform-methanol-water (70:30:4 by volume) which separates the neutral lipid, phosphatidylethanolamine, and monoacylglycerophosphorylethanolamine. Diglycerides which contained different numbers of double bonds in the acyl groups were separated on thin layer plates which were made with 5% AgNO₃ (weight per volume of H₂O). The developing system was chloroform-ethanol (95:5 by volume) (10). The products were made visible by staining with I₂ vapor and their radioactivity was determined by counting the compound on silica acid in a scintillation fluid composed of toluene, Triton X-100, and water in a ratio of 2:1:0.2 (by volume). Protein was determined by the method of Lowry et al. (11).

Materials—The labeled phospholipids were prepared as described earlier (8). The 2-[1-14C]linoleoyl 1-acylglycerol was prepared by the hydrolysis of 2-[1-14C]linoleoyl lecithin by phospholipase C (EC 3.1.4.3) from Bacillus cereus (12). Passage of 2-[1-14C]linoleoyl 1-acylglycerol through a column of silica acid in chloroform caused about 40% of the acyl group to migrate to position 3. The 3-[1-14C]linoleoyl 1-acylglycerol was separated from the 1,2 isomer by thin layer chromatography for use in the experiment described in Table IV. Both the 1,2 and 1,3 isomers were better than 90% pure. The 1- [9,10-3H₂]palmitoyl 2-arachidonylglycerol was prepared from 1-10,10-3H₂]palmitoyl 2-arachidonylglycerol by treatment with phospholipase C. The diglycerides were purified by thin layer chromatography and the species which contained arachidonic acid was separated from the other species by chromatography on AgNO₃ thin layer plates.

The 1-[1-14C]monolein was purchased from Applied Science Laboratories, Inc., Ann Arbor, Mich., and tr[1-14C]palmitoyl glycerol, [1-14C]linoleic, and [9,10-3H₂]oleic acid from Searle Corp., Des Plaines, Ill. The nonradioactive monoglycerides and diglycerides (all containing oleic acid) were purchased from Servadly Research Laboratories, Inc., London, Ontario, and were shown to be pure by thin layer chromatography. All labeled lipids were at least 95% radio pure, adjudged by thin layer chromatography. The specific activity of the 14H-labeled glycerides was 2.5 × 10⁵ cpm per μmole and of the 14C-labeled glycerides, 1.0 × 10⁴ cpm per μmole. The specific activity of the [1-14C]linoleic acid was 52 μCi per μmole, of the [9,10-3H₂]oleic acid, 450 μCi per μmole, and of the [U-14C]glycerol, 16 μCi per μmole. Heparin (170 units per mg) was purchased from Sigma, St. Louis, Mo.

RESULTS

We studied the substrate specificity of the phospholipase A₁ and found that in addition to phosphatidylethanolamine, monoacylglycerophosphorylethanolamine, 1-monoacylglycerol, and 1,2-diglyceride were hydrolyzed (Table I). The expected products of hydrolysis catalyzed by an enzyme specific for position 1 of the glycerides were labeled fatty acid from the phosphatidylethanolamine, monoacylglycerophosphorylethanolamine, and 1-monoacylglycerol, and labeled monoglyceride from the 1,2-diglyceride. If monoglyceride was present (or diglyceride, a monoacylglycerol precursor), radioactive diglyceride was also formed, the result of transesterification of the acyl group from a radioactive donor molecule to an acceptor monoglyceride. Triton X-100 decreased the transacylation drastically and to a lesser extent, reduced hydrolysis (with the exception of diglyceride whose hydrolysis was stimulated). The 14C-labeled 1,2-diglyceride formed both 14C-monoglyceride and 14C-labeled free fatty acid; the hydrolysis of the 14C-fatty acid probably occurred after migration of the fatty acid from position 2 to 1. In control experiments we found that about 50% of the 1,2-diglyceride was rapidly and nonsymatically transformed to 1,3-diglyceride. The acyl migration was reduced in the presence of Triton X-100. The reduction in the amount of 14C-fatty acid produced from 14C-diglyceride is the result of inhibition of the migration of the 14C-fatty acid by Triton X-100. The utilization of 1-monoacylglycerophosphorylethanolamine was reduced about 4-fold by phosphatidylethanolamine and monoglyceride and about 2-fold by diglyceride. The 1-monoacylglycerophosphorylethanolamine could serve as an acyl donor but not as an acyl acceptor since no radioactive phosphatidylethanolamine was formed. Triglyceride was not formed by this system, and triglyceride was very poorly hydrolyzed, even in the presence of Triton X-100 and defatted bovine serum albumin (not shown).

It is possible that the transacylation could be the result of reacylation of the hydrolyzed fatty acid. If reacylation occurs, radioactive free fatty acid should be incorporated into diglyceride. When [3H]oleic acid was added with 14C-monoglyceride as acyl

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<th>Product</th>
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<td></td>
<td></td>
<td>FA</td>
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<tr>
<td>1. 1-[9,10-3H₂]PE</td>
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<td>2. 1-[9,10-3H₂]MAGPE</td>
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<td>8.8</td>
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<tr>
<td></td>
<td>-</td>
<td>1.9</td>
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<tr>
<td>3. 1-[1-14C]MG</td>
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<tr>
<td>4. 2-[1-14C]DG</td>
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<td>0.9</td>
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*MG, monoglyceride; DG, diglyceride; FA, fatty acid; PE, phosphatidylethanolamine; MAGPE, monoacylglycerophosphorylethanolamine.
acceptor with or without nonradioactive phosphatidylethanolamine (Table II, Parts 1 and 2) only"C-diglyceride was produced. Likewise, "C-linoleic acid was not incorporated when assayed with ["H]phosphatidylethanolamine as acyl donor and nonradioactive acceptor monoglyceride (Part 3). In experiments not shown here, we found that the addition of ATP, coenzyme A, and Mg2+ had no effect on the utilization of free fatty acid. Furthermore, we could find no evidence that free [U-"C]glycerol was utilized. These results eliminate the possibility that transacylation is a combination of hydrolysis and reacylation. In this experiment we also separated the 1,2-diglyceride from the 1,3-diglyceride in order to determine the positional specificity of the acyl acceptor hydroxyl groups. With either phosphatidylethanolamine and monoglyceride as acyl donors and 1-monoglyceride as acceptor both positions 2 and 3 were acylated.

We would expect during the course of these reactions a precursor-product relationship between the original substrate (monoglyceride or phosphatidylethanolamine), the intermediate (diglyceride), and the final product (free fatty acid). As shown in Fig. 1a, diglyceride was the main product from "C-monoglyceride for 15 min only. As the reaction proceeded and as "C-monoglyceride was utilized, "C-diglyceride was then hydrolized and the major product was "C-fatty acid. Similar results were obtained when no phosphatidylethanolamine was added (results not shown), which again shows that phosphatidylethanolamine has little effect on the utilization of monoglycerides (cf. Fig. 1 and Table I). With ["H]phosphatidylethanolamine as the acyl donor (Fig. 1, b and c), the accumulation of "H-diglyceride occurs for 15 min only, even though the production of "H-fatty acid continues for at least 60 min. Since much less ["H]phosphatidylethanolamine has been utilized than under comparable conditions with "C-monoglyceride, it was not surprising that there was no decrease in "H-diglyceride at the later times of incubation (compare a with b). When diglyceride was substituted for monoglyceride as acyl acceptor (Fig. 1c), less "H-diglyceride was recovered and the rate of production of "H-fatty acid decreased after the first 15 min. Utilization of "C-diglyceride alone (results not shown) produced "C-monoglyceride, which decreased with time as transacylation and hydrolysis of the monoglyceride proceeded. If monoglyceride replaced phosphatidylethanolamine (Fig. 1f) more "C-monoglyceride and less "C-fatty acid was produced probably owing to the dilution of the product, "C-monoglyceride.

Fig. 2 (bottom) shows that the ratio of 1,2- to 1,3-diglyceride produced from 1-"C-labeled 1-monoglyceride is constant, which suggests that both products are formed by the enzyme rather than one product being formed by the enzyme and the second arising via acyl migration of the enzymatic product. This is supported by the results in the middle and top of the figure which shows that Triton X-100 (which decreased acyl migration) has no effect on the ratio of the two isomers of diglyceride recovered. The ratio of the isomers varies somewhat from experiment to experiment (cf. Table II and Fig. 2) for reasons not yet understood.

The utilization of monoglyceride as acyl acceptor and donor was studied (Fig. 3) with varying concentrations of the substrate and a fixed concentration (50 pm) of acyl donor phosphatidylethanolamine (a), and with a fixed concentration of substrate (50 pm) and variable concentrations of phosphatidylethanolamine (b). At all concentrations, phosphatidylethanolamine inhibited the total activity and production of diglyceride from monoglyceride (Fig. 3) while hydrolysis was not affected below 50 pm phosphatidylethanolamine. Below 50 pm monoglyceride (plus 50 pm phosphatidylethanolamine) hydrolysis predominated; above this concentration no more free fatty acid was recovered while the amount of diglyceride formed increased up to 200 pm mono-

glyceride.

Increasing concentration of both acyl acceptor and donor 1- and 2-monoglyceride inhibited the production of fatty acid from acyl donor 1-"C-phosphatidylethanolamine to an equal extent (Fig. 4). The production of diglyceride, however, was dependent upon the position of the acyl group in the monoglyceride. Over twice as much 1,2-diglyceride was produced when 2-acylmonoglyceride served as the acyl acceptor of the "C-fatty acid of phosphatidylethanolamine, as compared with 1-acylmonoglyceride as the acyl acceptor. With both isomers of monoglyceride considerably less 1,3-diglyceride was formed. These results suggest that the enzyme can acylate either position 2 or 3 of monoglyceride but prefers position 3. We cannot make a definitive statement on the positional specificity, however, due to acyl migration.

In a similar study (Fig. 5) both 1,2-diglyceride and 1,3-diglyceride decreased the production of "C-fatty acid from acyl donor 1-"C-phosphatidylethanolamine (c). About 4 times as much "C-diglyceride (c) was produced with added 1,2-diglyceride than with 1,3-diglyceride. The radioactive diglyceride is produced by hydrolysis of the nonradioactive diglyceride to monoglyceride followed by transacylation of the radioactive fatty acid from donor phosphatidylethanolamine to the acceptor monoglyceride. It is possible that the difference in the production of radioactive diglyceride is the result of poor hydrolysis of the 1,3-diglyceride. This proved not to be the case, however, since Table III shows that more 1,3-diglyceride was hydrolyzed than the 1,2-isomer. The difference in the radioactive diglyceride production seen in Fig. 5 must be the result of the preferential
**Fig. 1.** Utilization of 2-\(^{14}C\)-labeled 2-diglyceride, 1-[\(9,10\)-\(^{3}H\)]phosphatidylethanolamine, and 1-\(^{14}C\)-labeled 1-monoglyceride as a function of time. Each substrate was incubated with 44 \(\mu\)g of protein for the indicated lengths of time with the designated lipid (50 \(\mu\)M) added as described under "Experimental Procedures." MG, monoglyceride; PE, phosphatidylethanolamine; FA, fatty acid; DG, diglyceride.

**Fig. 2 (left).** The influence of Triton X-100 on the production of 1,2- and 1,3-diglyceride (DG) and free fatty acid (FFA) from 1-\(^{14}C\)-labeled 1-monoglyceride. The incubations were run for the indicated periods of time and concentrations of Triton X-100 using 42 \(\mu\)g of protein, as described under "Experimental Procedures."

**Fig. 3 (center).** The effect of the addition of phosphatidylethanolamine on the utilization of 1-\(^{14}C\)-labeled 1-monoglyceride. Top, increasing concentrations of monoglyceride were used as substrate with 50 \(\mu\)M phosphatidylethanolamine and 44 \(\mu\)g of protein as described under "Experimental Procedures." Bottom, 50 \(\mu\)M 1-\(^{14}C\)-labeled 1-monoglyceride was assayed with increasing concentrations of phosphatidylethanolamine. See Fig. 1 for abbreviations.

**Fig. 4 (right).** The effect of 1- and 2-monoglyceride (MG) on the utilization of 1-[\(1\)-\(^{14}C\)]phosphatidylethanolamine. The indicated concentrations of the monoglycerides were added to reaction mixtures which contained 50 \(\mu\)M 1-[\(1\)-\(^{14}C\)]phosphatidylethanolamine with 42 \(\mu\)g of protein and incubated as described under "Experimental Procedures." DG, diglyceride; FFA, free fatty acid.
The production of radioactive 1,2-diglyceride. This is contained 50 μg of protein incubated as described under "Experimental Procedures." The symbols for the products are total (sum of fatty acid and diglyceride, A), fatty acid (v), and diglyceride (D).

Direct evidence that diglyceride can act as an acyl donor in transacylation is provided by the demonstration that the addition of 2-14C-labeled 1,2-diglyceride can act as an acyl donor in transacylation of acceptor monoglyceride with 3H-labeled 1,3-diglyceride, presumably via transacylation. Direct evidence that diglyceride can act as an acyl donor in transacylation is found in Table IV and Fig. 6. As shown before, 1-[14C]monolein gave rise primarily to radioactive diglyceride. Acyl donor diglyceride which contains [3H]palmitic acid in position 1 and nonradioactive arachidonic acid in position 2 was hydrolyzed by the enzyme to [3H]palmitic acid primarily. However, when the two were together as substrates, the diglyceride recovered contained an equal amount of 3H and 14C. To show that this was not simply inhibition of 3H-diglyceride hydrolysis and uninhibited transacylation of 14C-monoglyceride but the result of the transfer of 14C fatty acid from an acyl donor diglyceride to an acceptor [14C]monolein, we chromatographed the isolated diglycerides on thin layer plates which contained AgNO3 in order to separate the diglycerides according to the numbers of double bonds in the molecule. The substrate 3H-diglyceride contained four double bonds (arachidonic and palmitic acids) and the product of 14C-monoglyceride transacylation contained two double bonds (two oleic acids) whereas the product of transacylation of the fatty acid from position 1 of 3H diglyceride to 14C monoglyceride gives rise to a product that had an equal amount of 3H and 14C and one double bond (palmitic and oleic acids). Fig. 6 shows that the diglyceride recovered from the reaction mixture with 3H-diglyceride (bottom) as the sole substrate contains four double bonds (Fraction 4); the diglyceride from 14C-monoglyceride (top) contains two double bonds (Fraction 5); and that the two substrates added together as substrates gave as product a diglyceride that ran in between diolen and diplimathin (Fraction 6), the location expected of a diglyceride with one double bond. This shows that the fatty acid from position 1 of diglyceride can be transferred to an acceptor monoglyceride and that this appears to be a favored reaction.

In previous work (8) we found that the enzyme freed from the membrane was stimulated 2-fold only by Ca2+. We speculated that the presence of the membranous lipids had influenced the requirement for Ca2+. Fig. 7 shows that the effect of Ca2+ on the enzymatic activity was dependent on the lipids present. As reported (8), the hydrolysis of [3H]phosphatidylethanolamine (a) was stimulated only 2- to 3-fold by Ca2+ When diglyceride was added (b), a 2-fold stimulation of hydrolysis is noted at 1 to 2 mM Ca2+. Higher concentrations of Ca2+ shifted the activity toward transacylation, possibly the result of increased hydrolysis of diglyceride (also stimulated by Ca2+; Fig. 7, f and g) which produced the acyl acceptor monoglyceride. Interestingly, when 1-acyl acceptor monoglyceride was added (c) both hydrolysis and transacylation of phosphatidylethanolamine require Ca2+. The transacylation of monoglyceride (acyl acceptor and donor) with added phosphatidylethanolamine (e) was also highly dependent on Ca2+ which was not the case if phosphatidylethanolamine was omitted (d). The hydrolysis of monoglyceride was unaffected by Ca2+ in both cases. Ca2+ increased the hydrolysis of diglyceride (f and g) roughly 2-fold in all cases.

Further examination of the characteristics of the hydrolytic and transacylation activities of this system revealed that the hydrolysis of both monoglyceride and phosphatidylethanolamine

\[ \text{TABLE III} \]

**Utilisation of 1,β- and 1,δ-diglyceride by the phospholipase A1**

Both substrates were assayed with 42 μg of solubilized enzyme either singly or with lipid added (50 μL), as designated below. The incubation conditions are described under "Experimental Procedures."

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<thead>
<tr>
<th>Substrate</th>
<th>MG*</th>
<th>Changes in compound recovered</th>
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<td></td>
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<td>mg</td>
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<tr>
<td>1,3-[3-14C]DG</td>
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</tr>
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</table>

*MG, monoglyceride; DG, diglyceride; FA, fatty acid.

\[ \text{TABLE IV} \]

**Utilization of 1-14C-labeled 1-monoglyceride and 9,10-3H2-labeled 1-diglyceride by phospholipase A1**

The 1-14C-labeled 1-monoglyceride (50 μM) and the 9,10-3H2-labeled 1-diglyceride (28 μM) were assayed with 42 μg of solubilized enzyme singly and in combination as described under "Experimental Procedures." The lipids were separated by thin layer chromatography and were eluted from the silicic acid with CHCl3. One-quarter of each fraction was counted in a scintillation counter.

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<th>Substrate</th>
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<tr>
<td></td>
<td>mg</td>
</tr>
<tr>
<td>1-[14C]MG, 1-δ-DG, 1,δ-DG</td>
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<td>1.5 21.6</td>
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*MG, monoglyceride; DG, diglyceride; FA, fatty acid.
FIG. 6. Thin layer chromatography of the diglycerides (DG) obtained in Table IV. Three-quarters of the CHCl₃ solutions described in the legend of Table IV which contained diglyceride were chromatographed on thin layer plates which contained AgNO₃ as indicated under "Experimental Procedures." The areas corresponding to the following standards were scraped and counted in a scintillation counter, 1, oleic acid and monoolein; 2, palmitic acid; 3, open; 4, dilinolein; 5, diolein; 6, open; 7, dipalmitin; 8, open. MG, monoglyceride.

FIG. 7. The effect of Ca²⁺ on the utilization of ¹⁴C-labeled 2-diglyceride (DG), I-¹⁴C-labeled phosphatidylethanolamine (PE), and ¹³C-labeled 1-monoglyceride (MG). The assays were run using 44 µg of protein as described under "Experimental Procedures," except for the indicated changes in Ca²⁺ concentration. FA, fatty acid.

DISCUSSION

We have provided evidence in this communication that the phospholipase A₁ of the plasma membrane of rat liver is capable of hydrolyzing and transacylating the following substrates: 1, oleic acid and monoolein; 2, palmitic acid; 3, open; 4, dilinolein; 5, diolein; 6, open; 7, dipalmitin; 8, open. MG, monoglyceride.
of catalyzing the transfer of the fatty acid from position 1 of phosphatidylethanolamine, monoaoylglycerophosphorylethanolamine, monoglyceride or diglyceride to the acceptor hydroxyl of water (a hydrolytic reaction) or of monoglyceride (a transacylation reaction). Since monoglyceride seems to be the preferred substrate and since the production of diglyceride appears to be the major product, the enzyme might be more appropriately named monoglyceride acyltransferase. However, before such a name is adopted, it should be demonstrated that the enzyme, once purified to homogeneity, has all these activities.

Vogel et al. (9) demonstrated that glycerol (as well as methanol and ethanol) could act as an acyl acceptor for the fatty acid removed from phosphatidylethanolamine by the post-heparin serum phospholipase. They used much higher concentrations of glycerol (12 to 50%), than used by us (5 mM). Comparison of their results with ours shows that the availability to the enzyme of the hydroxyl group of the acceptor molecule determines which product (ester or free fatty acid) will be formed. Triton X-100 which led to the disruption to the liposome and increased availability of water to the enzyme shifted the system toward the production of free fatty acid.

The hydrolysis of monoglyceride and diglyceride by the phospholipase A₁ solubilized from the plasma membrane confirms the observation of Zieve et al. (13) who showed that the phospholipase A₁ which was partially purified from serum was capable of hydrolyzing these substrates. Earlier, Greten (14) found that activities that catalyze the hydrolysis of lecithin and monoglyceride were released into the serum at the same time after the injection of heparin which he took as evidence that the two activities are catalyzed by the same enzyme. We believe that the main reason that the other workers have not observed the transacylation activity with monoglyceride is because their assay medium contained a detergent which inhibits transacylation or that the only product measured was free fatty acid.

The triglyceride lipoprotein lipase was shown to be distinct from the phospholipase by Greten et al. (15) who found that the two activities had different sensitivities to heat and inhibitors such as NaCl and protamine. In certain cases we were able to find hydrolysis of triglyceride by heparin-solubilized fraction from the plasma membranes, in confirmation of the report of Assmann et al. (16). However, such activity was absent in some preparations and, in preparations which were active, lost the ability to attack triglyceride upon storage while retaining the other activities reported in this paper.

Thus far, the only glyceride we have been able to demonstrate to be an acyl acceptor is monoglyceride. The evidence obtained thus far indicates that either position 2 or 3 can accept the acyl group. We have not found any utilisation of 2-acylglycerophosphorylethanolamine (produced by the hydrolysis of phosphatidylethanolamine in the system) as an acyl acceptor. This suggests that a compound with two hydroxyl esterified cannot act as an acyl acceptor. The enzyme appears to have a specificity for the donor molecule that is different than that for the acceptor molecule. The acyl donor can have two hydroxyl esterified, and we find only the ester of a primary alcohol can be removed, although it must be pointed out that we have not yet tested
2-acylmonoglyceride as an acyl donor. On this basis we suggest that the enzyme has two binding sites for substrates, one for the donor and one for the acceptor molecule. The fact that an acceptor molecule such as 2-acylmonoglyceride can reduce the utilization of a donor molecule such as phosphatidylethanolamine can be explained by changes in the liposome which lead to the dilution of phosphatidylethanolamine in the liposome, thereby reducing the effective substrate concentration. The reduction in utilization of a donor molecule by another donor molecule could be the result of such a dilution of substrate in addition to a competitive type inhibition. Based on the finding that free fatty acid is not incorporated into glyceride we believe that the acyl group remains tightly bound to the enzyme during transacylation, probably through a covalent ester linkage, similar to the conclusion reached by Vogel et al. (9).

The discovery of the transacylation activity raises interesting questions concerning its possible physiological role. If the enzyme functions mainly in the plasma membrane, it could, together with the lipoprotein lipase, account for the hydrolysis of triglyceride of circulating lipoproteins and facilitate absorption by the liver. One mechanism could be an initial hydrolysis of the triglyceride by the triglyceride lipase followed by a secondary attack by the phospholipase A1 to produce monoglyceride and free fatty acid, both of which are then able to cross the plasma membrane. However, it is conceivable that the enzyme could itself carry the acyl group through the membrane and catalyze the transacylation on the inside of the cell to re-form the diglyceride. Energetically, this transport mechanism would be of advantage to the cell in the uptake of triglyceride since only one acyl-CoA would be utilized in the reconstruction of the triglyceride. Phosphatidylethanolamine also could be taken up by a similar mechanism in which the acyl group would be transferred to a neutral glyceride and the other product, monoacylglycerophosphoryl ethanolamine, would then be the acceptor for a new acyl group from acyl-CoA. The role of plasma membranes in esterification is still unclear (17) although it has been shown that monoacylglycerophosphorylcholine can be taken up by the liver (18). This would provide a mechanism which could serve as an alternative to the uptake of intact phospholipids by the liver (19). If the phospholipase A1 was released by heparin into the blood it might catalyze the same types of reactions within the circulating lipoproteins, perhaps involving transacylation between different lipoproteins.

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Utilization of Neutral Glycerides and Phosphatidylethanolamine by the Phospholipase A₁ of the Plasma Membranes of Rat Liver

Moseley Waite and Patricia Sisson


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